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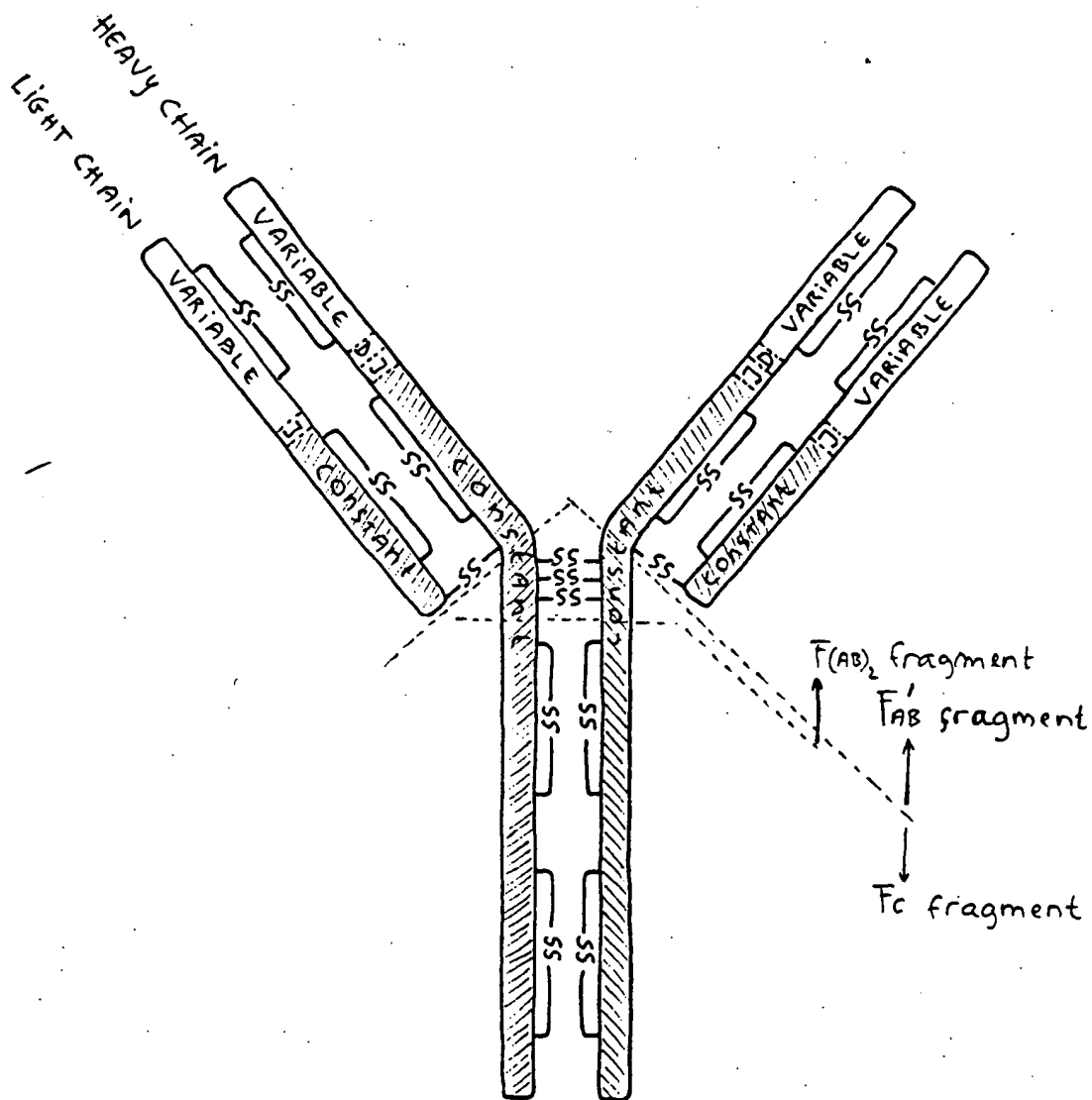


FIGURE 1

Schematic representation of a typical immunoglobulin

1 GTTGTCTGG TTGTCTGGT TTGAAGGAGA CATTGTGATG ACCCAGTCTC ACAAATTCAT GTCCACATCA GTAGGAGACA GGGTCAGCAT CACCTGCAAG  
CAACGACACC AACAGACCAC AACTTCTCT GTAACACTAC TGGGTAGAG TGTAAAGTA CAGGTAGT CATCTCTGT CCCAGTCGTA GTGGACGTTT  
haeIII haeI

101 GCCAGTCAGG ATGTGGGTGC TGCTATAGCC TGGTATCAAC AGAAACCAGG ACAATCTCCT AAACACTGA TTTACTGGG ATCCACCCGG CACACTGGAG  
CGGTAGTCC TACACCCACG ACGATATCGG ACCATAGTTG TCTTTGGTCC TGTAGAGGA TTTGATGACT AAATGACCCG TAGGTGGGCC GTGTGACCTC  
fokI sfaNI

201 TCCCTGATCG CTTACAGGC AGTGGATCTG GGACAGATT CACTCTCACC ATTAGCAATG TGCAGTCTGA TGACTTGGCA GATTATTCT GTCAACAATA  
AGGACTAGC GAAGTGTCCG TCACCTAGAC CCTGTCTAAA GTGAGAGTGG TAATCGTTAC ACGTCAGACT ACTGAACCGT CTAATAAAGA CAGTTGTAT  
sau3A xhoI  
dnlI sau3A  
dnlI hphI hincII

301 TAGCGGGTAT CCTCTCACGT TCGGTGCTGG GACCAAGCTG GAGCTGAAAC GGGCTGATGC TGCACCAACT GTATCCATCT TCCACCATC CAGTGAGCAG  
ATCGCCATA GGAGAGTGCA AGCCACGACC CTGGTTCGAC CTCGACTTTC CCCGACTACG ACGTGGTTGA CATAGGTAGA AGGGTGGTAG GTCACTCTGC  
mnII sau96  
fnu4HI hpaI  
hincII

401 TTAACATCTG GAGGTGCCTC AGTCGTGTGC TTCTTGAACA ACTTCTACCC CAAAGACATC AATGTCAAGT GGAAGATTGA TGGCAGTGAA CGACAAAATG  
AATTGTAGAC CTCCACGGAG TCAGCACACG AAGAAGTGT TGAAGATGG GTTCTGTAG TTACAGTTCA CCTCTAAT ACCGTCACCT GGTGTTTTAC  
mnII ddel xhoI mbolI acyl

501 GCGTCCTGAA CAGTTGGACT GATCAGGACA GCAAAGACAG CACCTACAGC ATGAGCAGCA CCCTCACGTT GACCAAGGAC GAGTATGAAC GACATAACAG  
CGCAGGACTT GTCAACCTGA CTAGTCTGT GGTTCCTGTC GTGGATGTGC TACTCGTCTG GGGAGTGCAA CTGGTTCCTG CTCATACTTG CTGTATTGTC  
sau3A  
dnlI hpaI hincII

601 CTATACCTGT GAGGCCACTC ACAAGACATC AACTTCACCC ATTGTCAAGA GCTTCAACAG GAATGAGTGT TAGAGACAAA GGTCTGAGA CGCCACCACC  
GATATGGACA CTCCGGTGAG TGTCTGTAG TTGAAGTGGG TAACAGTTCT CGAAGTTGTC CTTACTCACA ATCTCTGTTT CCAGGACTCT GCGGTGGTGG  
mnII haeIII sau96 hgaI  
haeI hphI aluI  
dnlI acyl

701 AGCTCCCCAG CTCCATCCTA TCTTCCCTTC TAAGGTCTTG GAGGCTTCCC CACAAGCGAC CTACCACTGT TGCAGTCTC CAAACCTCCT CCCACCTCC  
TCGAGGGGTC GAGGTAGGAT AGAAGGGGAG ATTCCAGAAC CTCCGAAGGG GTGTTGCTGT GATGGTGACA ACGCCACGAG GTTTGGAGGA GGGGTGGAGG  
fokI

801 TTCTCTCTCT CTCCCTTTC CTGGCTTTT ATCATGCTAA TATTTGCAGA AAATATTCAA TAAAGTGAGT CTTTGCATT GA  
AAGAGGAGGA GGAGGGGAAA GAACCGAAAA TAGTACGATT ATAAAGTCT TTTAAGTT ATTTACTCA GAAACGTGAA CT  
mnII hincII xhoI hpaI

nucleotides: 882

FIGURE 2

Nucleotide sequence of pK17G4 cDNA insert, including  
~~light (non-coding) region.~~

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 CTGAGTCGTG ACTTGTCCT GGGGAGTGT ACTTGAAGCC CGAGTCGAAC TAAATGGAAC AGGAACAAAA TTTTCAACAG GTCACACTTG ATACGCTG

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 hinfI hinfI ecorII avall fnu4HI hinfI  
 GGAGTCCTGG GGAGTCTTAA TGGAGCCTGG AGGGTCCCTG AAACCTCTCT GTGCAGCCTC TGGATTCACT TTCAGTAGAT ATGCCATGTC TTGGGTTCGC  
 CCTCAGACCC CCTCAGAATT ACCTCGGACC TCCAGGGAC TTTGAGAGGA CACGTCGGAG ACCTAAGTGA AAGTCATCTA TACGGTACAG AACCCAAGCG

201  
 hpalI mnlI hinfI mbolI hinfI hpalI  
 CAGATCCCGG AGAAGAGGCT GGAGTGGGTC GCAACCATTG GTAGTGGTGG TAGTTCACAC CTTCATCCA GACAGTGTA AGGGCGATT ACCATCTCCA  
 GTCTGAGGCC TCTTCTCGA CCTCACCGAG CGTTGGTAAT CATCACCACC ATCAAGTGTG GAAGGTAGGT CTGTCACTCT TCCCGCTAAG TGGTAGAGGT  
 fokI

301  
 rsal mnlI mnlI ddel ddel haeIII  
 GAGACAATGC CAAGAACACC CTGTACCTGC AAATGAGCAG TCTGAGGTCT GAGGACACGG CCAATGTATTA CTGTGCAAGA CCCCCTCTTA TTTCTGTAGT  
 CTCTGTTACG GTTCTGTGG GACATGGAGC TTTACTGCTC AGACTCCAGA CTCTGTGCC GGTACATAAT GACACGTTCT GGGGGAGAAT AAGCAATCA

401  
 mnlI ddel hphI mnlI ddel haeIII  
 AGCGGACTAT GCTATGGACT ACTGGGGTCA AGGAACCTCA GTCAACGCTC CTCTAGCCAA AACGACACCC CCATCTGTCT ATCCACTGGC CCGTGGATCT  
 TCGCCTGATA CGATACCTGA TGACCCAGT TCTTGGAGT CAGTGGCAGA GGAGTCGGT TTGCTGTGGG GGTAGACAGA TAGGTGACCG GGGACCTAGA

501  
 fnu4HI ncoI sfaNI fokI hphI ecorII scrFI haeIII xhoII sau3A  
 GGTGCCCCAA CTAATCCAT GGTGACCTGG GGATGCTGG TCAAGGGCTA TTTCCCTGAG CCAGTGACAG TGACCTGGAA CTCTGGATCC CTGTCCAGCG  
 CGACGGGTTT GATTGAGTA CCACTGGGAC CTAACGACC AGTTCGCCAG AAAGGGACTC GGTCACTGTC ACTGGACCTT GAGACCTAGG GACAGGTCCG

601  
 hgIA pvuII aluI pstI mnlI ddel aluI fnu4HI sau96  
 GTGTGCACAC CTTCCAGCT GTCTGTCAGT CTGACCTCTA CACTCTGAGC AGCTCAGTGA CTGTCCCTC CAGCCCTCGG CCCAGCGAGA CCGTCACCTG  
 CACACGTGTG GAAGGGTCGA CAGGACGTCA GACTGGAGAT GTGAGACTCG TCGAGTCACT GACAGGGGAG GTCGGGAGCC GGGTGGCTCT GGCAGTGGAC

701  
 scrFI haeIII nciI fnu4HI hphI haeIII  
 CAACGTTGCC CACCGGGCCA GCAGCACCAA GGTGGACAAG AAAATTGTGC CAGGGGATTG TGGTTGTAAG CTTTGCATAT GTACAGTCCC AGAAGTATCA  
 GTTGCAACGG GTGGGCCGGT CGTCGTGGTT CCACCTGTT TTTTAAACAG GGTCCCTAAC ACCAACATTC GGAACGTATA CATGTCAGGG TCTTCATAGT

801  
 mboII mboII foki hgIA hphI hinfI mstII sau3A  
 TCTGTCTTCA TCTTCCCCC AAAGCCCCAG GATGTGCTCA CCATTACTCT GACTCTCAAG GTCACTGTG TTGTGGTAGA CATCAGCAAG GATGATCCCC  
 AGACAGAAGT AGAAGGGGGG TTTGCGGTTT CTACACGAGT GGTAAATGAGA CTGAGGATTC CAGTGACAC AACACCATCT GTAGTCGTTT CTACTAGGGC

901  
 sau96 pvuII mnlI ddel haeIII haeIII haeIII  
 AGTCCAGTT CAGCTGGTT GTAGATGATG TGGAGTGCA CACAGCTCAG ACACACCC GGGAGGAGCA GTTCAACAGC ACTTCCGCT CAGTCAGTGA  
 TCCAGGTCAA GTCGACCAA CATCTACTAC ACCTCCAGCT GTGTCGAGTC TCGTTGGGG CCGTCTCTGT CAAGTTGTCG TGAAGGGCGA GTCAGTCACT

1001  
 scrFI ecorII fnu4HI hincII haeIII haeIII haeIII  
 ACTTCCATC ATGCACAGG ACTGGCTCAA TGGCAAGGAG TTCAATGCA GGTCAACAG TGCAGCTTTC CCTGCCCCCA TCAGAGAAAAC CATCTCCAAA  
 TGAAGGGTAG TACGTGGTCC TGACCGAGTT ACCGTTCTCT AAGTTTACGT CCCAGTTGTC ACCTGCAAGG GACAGGGGGT AGCTCTTTTG GTAGAGGTTT

1101  
 rsal mnlI haeIII haeIII haeIII  
 ACCAAAGGCA GACCGAAGG TCCACAGGTG TACACATTC CACTCCCAA GGAGCAGATG GCCAAGGATA AAGTCAGTCT GACCTGCATG ATAACAGACT  
 TGGTTTCCGT CTGGCTCCG AGGTGTCCAC ATGTGGTAAG GTGGAGGGTT CCGTCTCTAC CGGTTCTTAT TTCACTCAGA CTGGACGTAC TATTGTCTGA

1201  
 mboII mboII fnu4HI haeIII haeIII haeIII  
 TCTTCCCTGA AGACATTACT GTGGAGTGGC AGTGAATGG GCAGCCAGCG GAGAACTACA AGAACACTCA GCCCATCATG AACACGAATG GCTCTTACTT  
 AGAAGGGACT TCTGTAATGA CACCTCACCG TCACCTTACC CACTGGTGGC CTCTGTGATG TCTGTGAGT CCGGTAGTAC TGTGCTTAC CGAAGATGAA

1301  
 accI aluI mboII mnlI hphI haeIII haeIII haeIII  
 CGTCTACAGC AAGCTCAATG TGCAGAAGAG CAATGGGAG GCAGGAAATA CTTTCACTG CTCTGTGTTA CATGAGGGCC TGCACAACA CCATCTGAG  
 GCAGATGTG TCGAGTTAC AGTCTTCTC GTTGACCTC CGTCTTTAT GAAAGTGGAC GAGACACAAT GTACTCCCGC ACCTGTTGGT GGTATGACTC

1401  
 mnlI scrFI sau3A mnlI hincII haeIII haeIII haeIII  
 AAGACCTCT CCCACTCTCC TGGTAAATGA TCCAGTGTG CTTGGAGCCC TCTGGTCTTA CAGGACTCTG ACACCTACCT CCACCTCTCC CTGTATAAT  
 TCTCGAGA GGGTGAAGG ACCATTACT AGGGTCACAG GAACCTCGGG AGACAGGAT GTCTGAGAC TGTGGATGA GGTGGGGAGG GACATATTTA

1501  
 AAAGCACCCA GCACTGCTT GGGAAAAA  
 TTTCTGGGT CGTGACGAA CCGTTTTT

FIGURE 4

p gamma 298 cDNA insert and p $\gamma$ 11 cDNA insert ligation containing coding sequence for heavy (gamma) anti CEA chain

FIGURE 5

Amino acid and coding sequence for heavy (gamma-1)  
anti CEA chain

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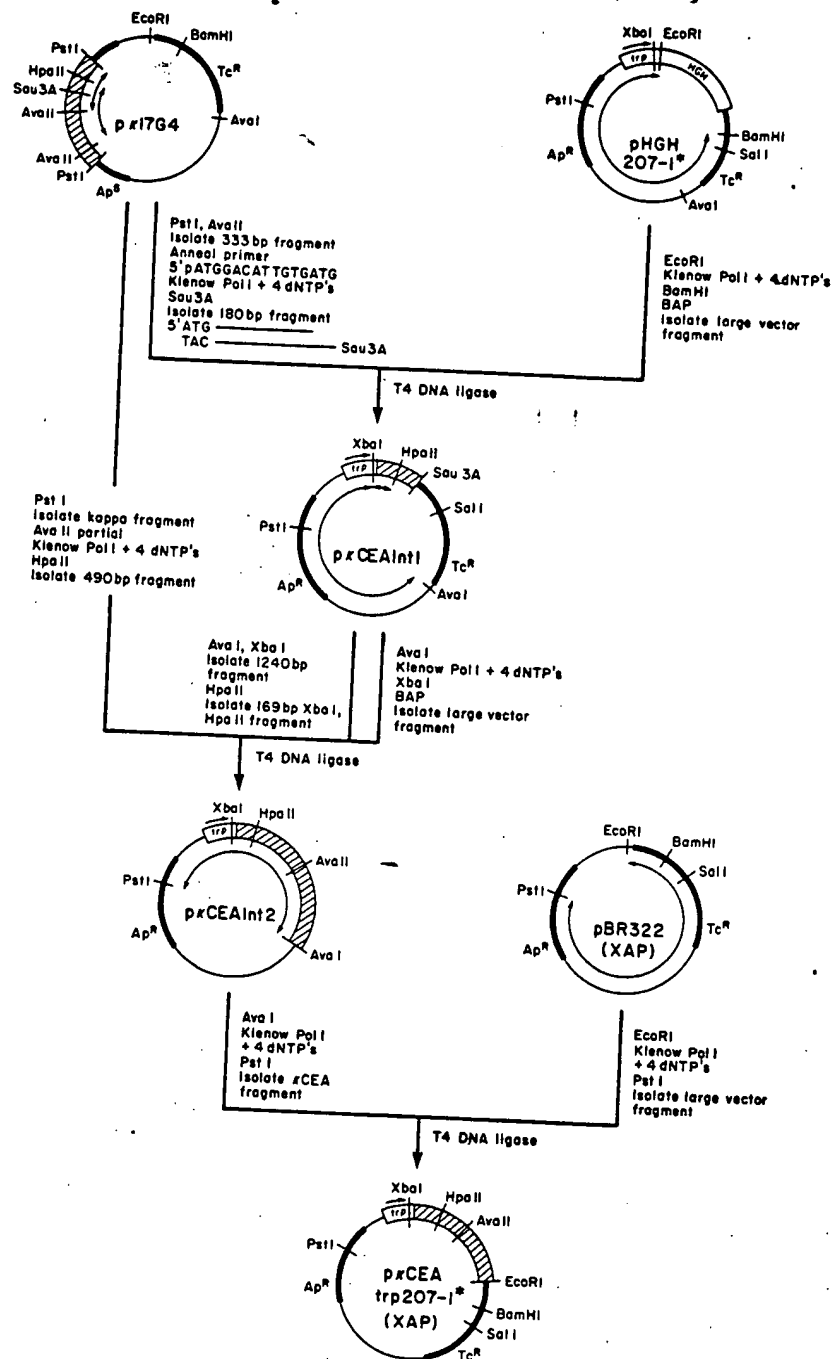


FIGURE 6

Construction of plasmid for direct expression of light anti CEA (kappa) chain gene

205419

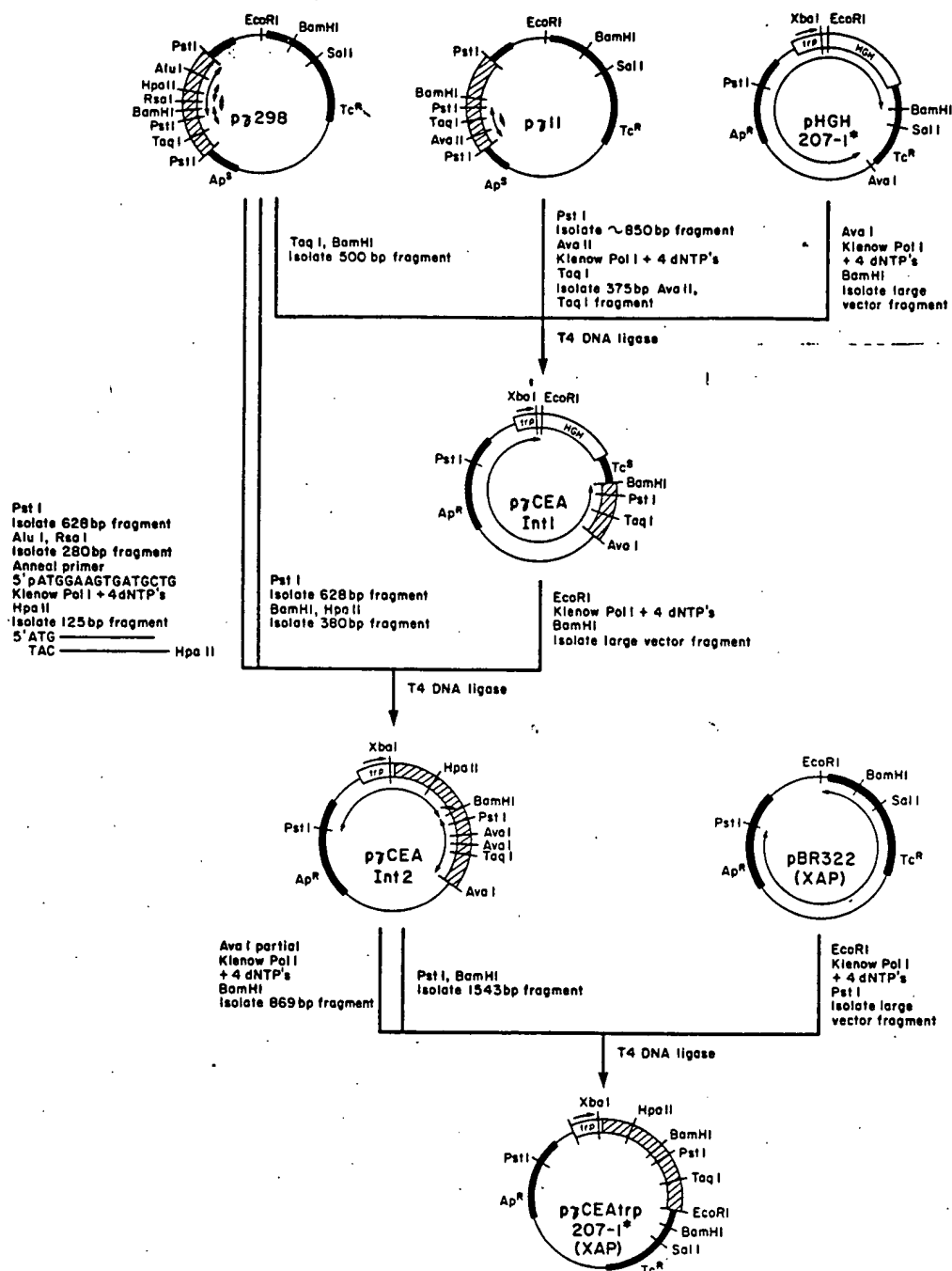


FIGURE 7

Construction of plasmid for direct expression of anti CEA light (gamma 1) chain

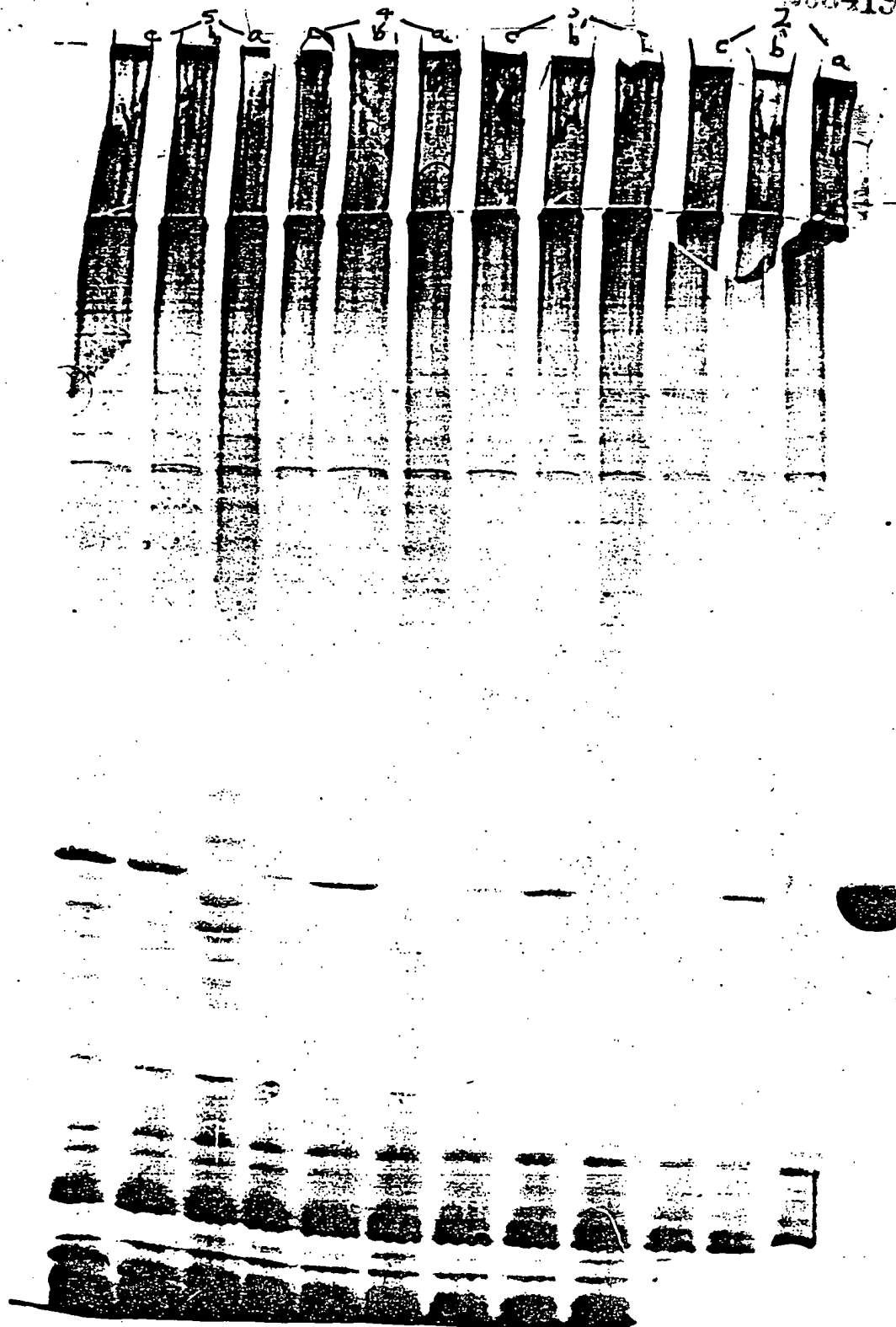


FIGURE 8A  
Silver stained SDS-PAGE of extracts from E. coli transformed with pYCEAt<sub>trp</sub>207-



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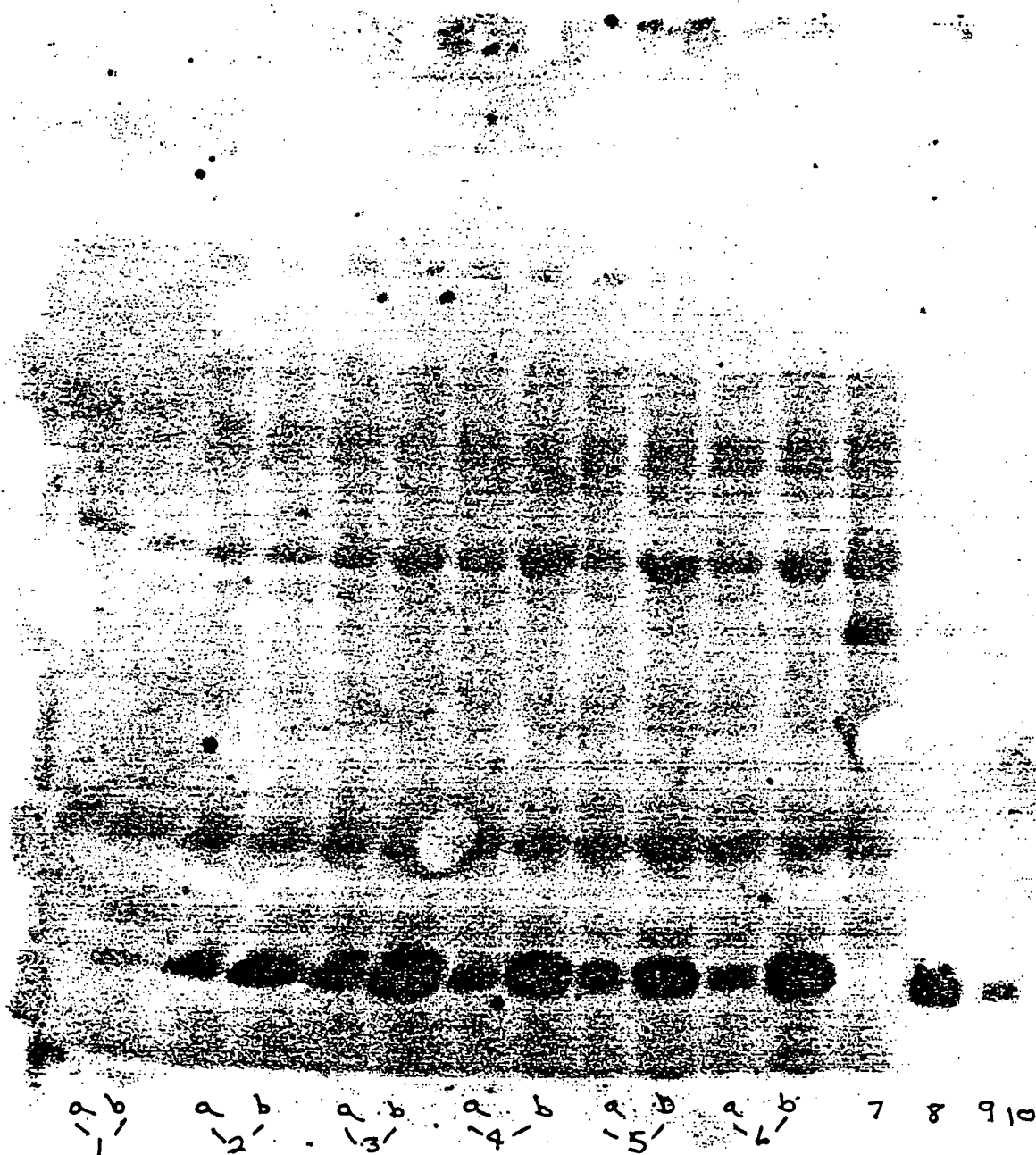


FIGURE 8B

Western blot of SDS PAGE performed on extracts of E.coli transformed with PKCEAtrp207-1\*

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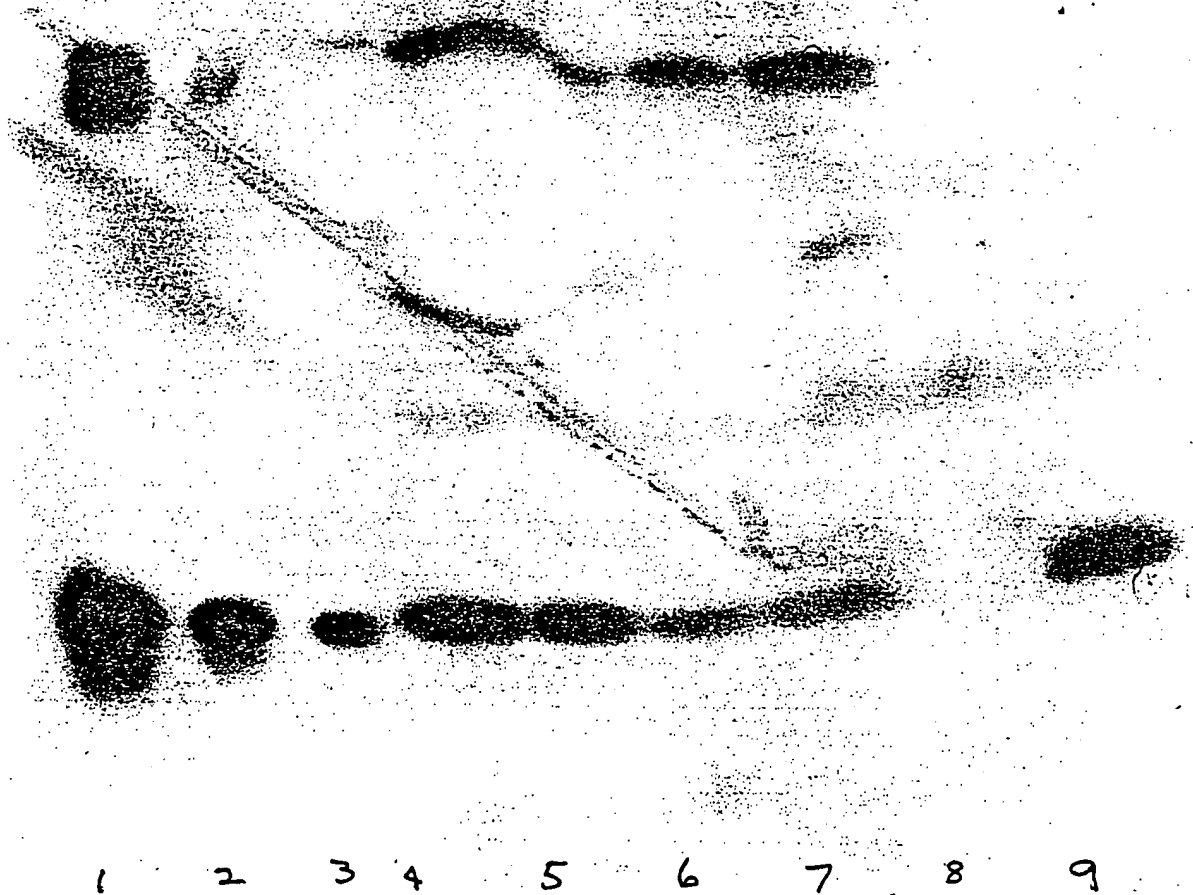
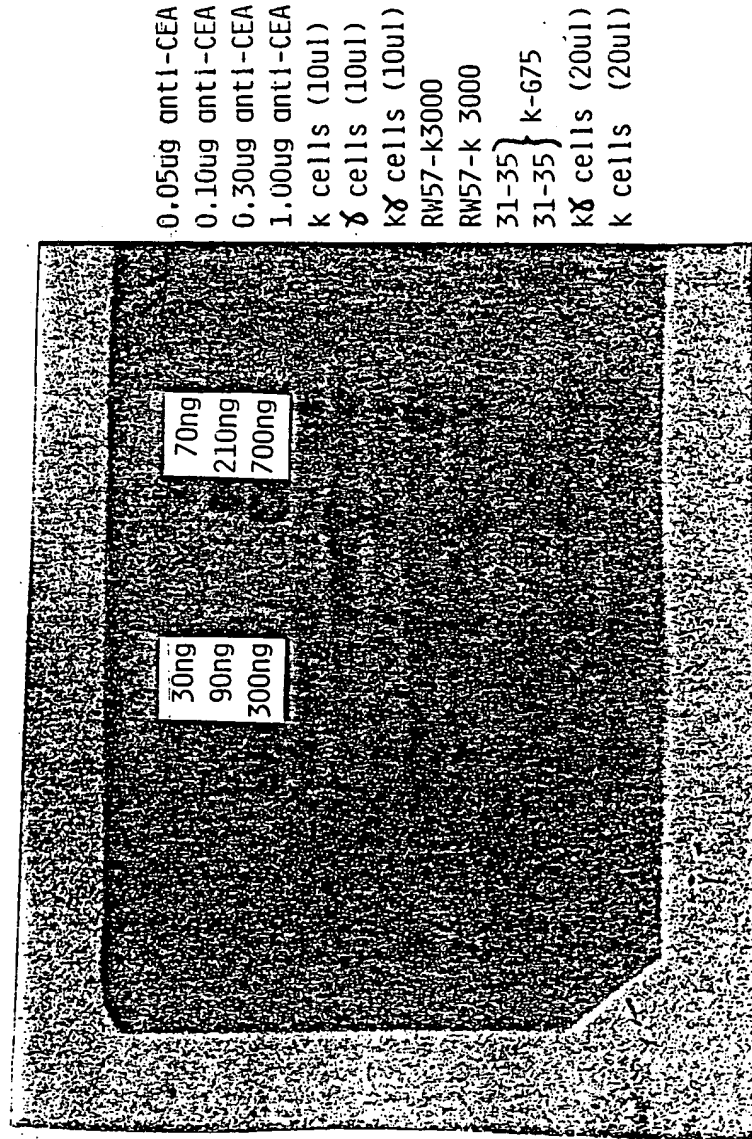


FIGURE 8C

Western blot of SDS PAGE E. coli double transformed with  
pKCEAtrp207-1\*4 and pyCEAInt2.

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0.05ug anti-CEA  
0.10ug anti-CEA  
0.30ug anti-CEA  
1.00ug anti-CEA  
k cells (10ul)  
k cells (10ul)  
k cells (10ul)  
RW57-k3000  
RW57-k 3000  
31-35 } k-675  
31-35 }  
k cells (20ul)  
k cells (20ul)

FIGURE 9

Western blot of SDS PAGE performed on extracts of E. coli transformed with plasmids containing DNA sequences and coding heavy light and both heavy and light anti CEA chains.

205419

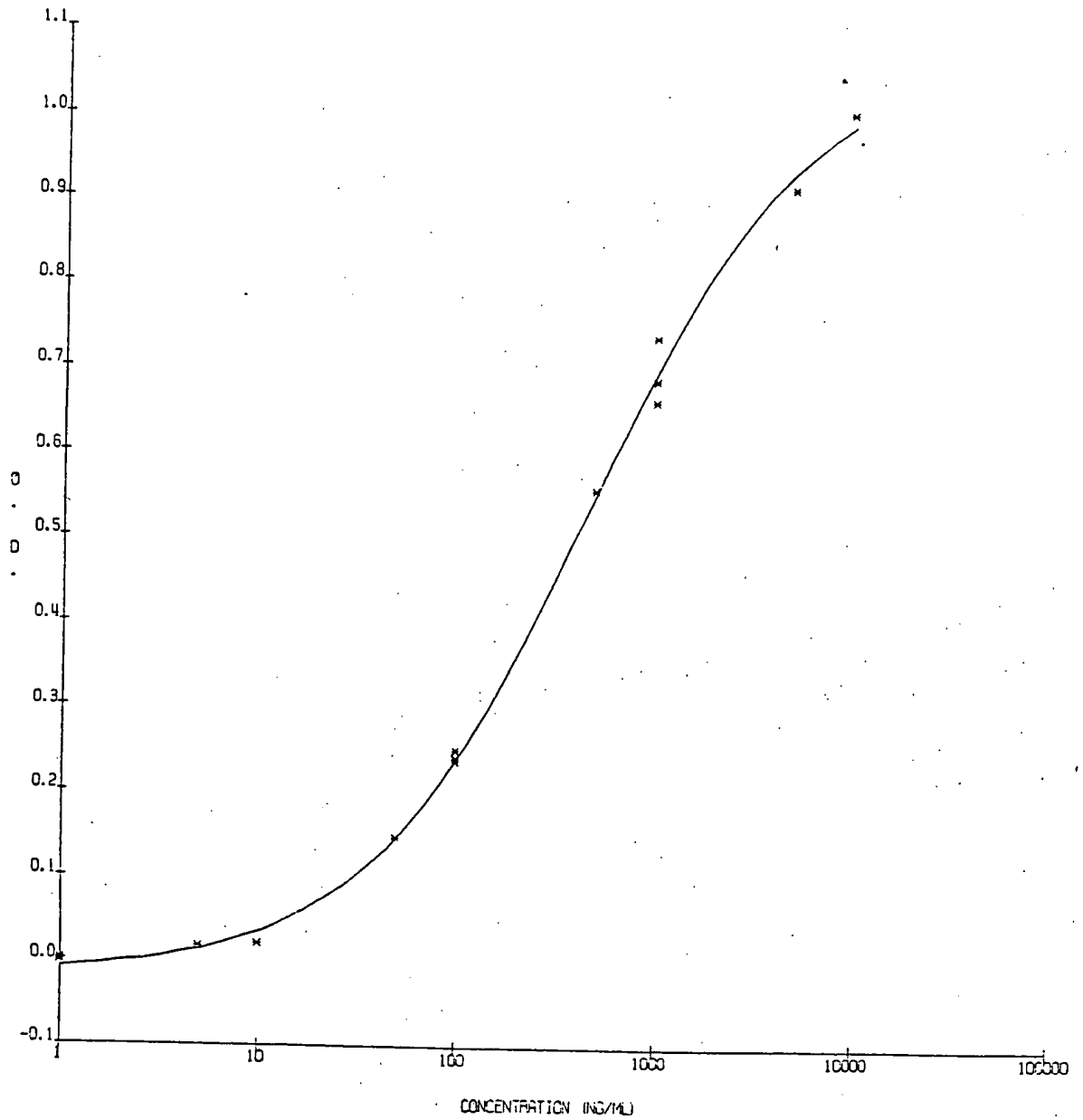


FIGURE 10

Standard curve showing dependence of OD on concentration of antibody by ELISA assay

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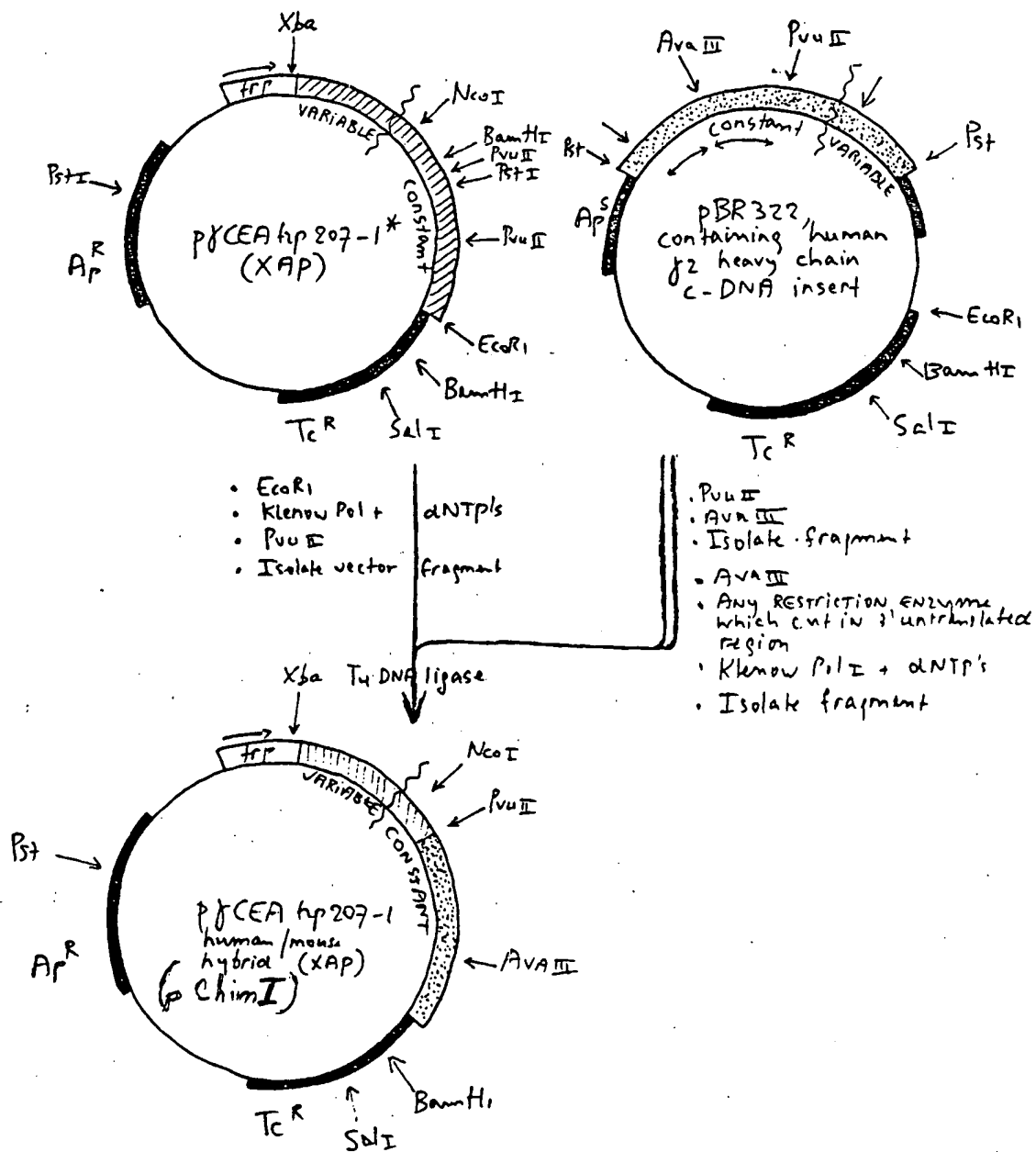


FIGURE 11

Construction of a plasmid for direct expression of chimeric human/murine heavy chain gene

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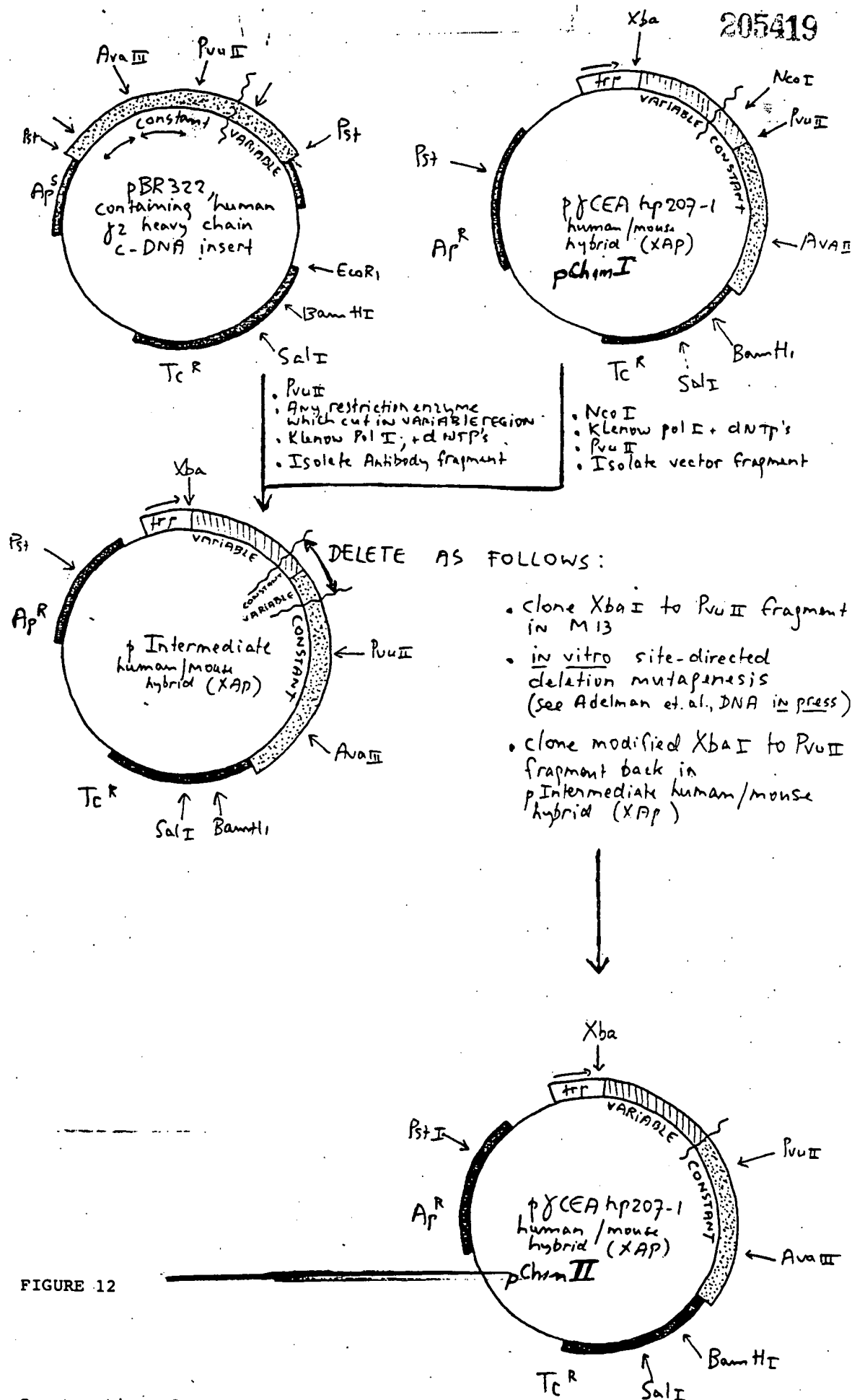


FIGURE 12

Construction of a plasmid for direct expression of chimeric constant/murine variable heavy chain gene

205419

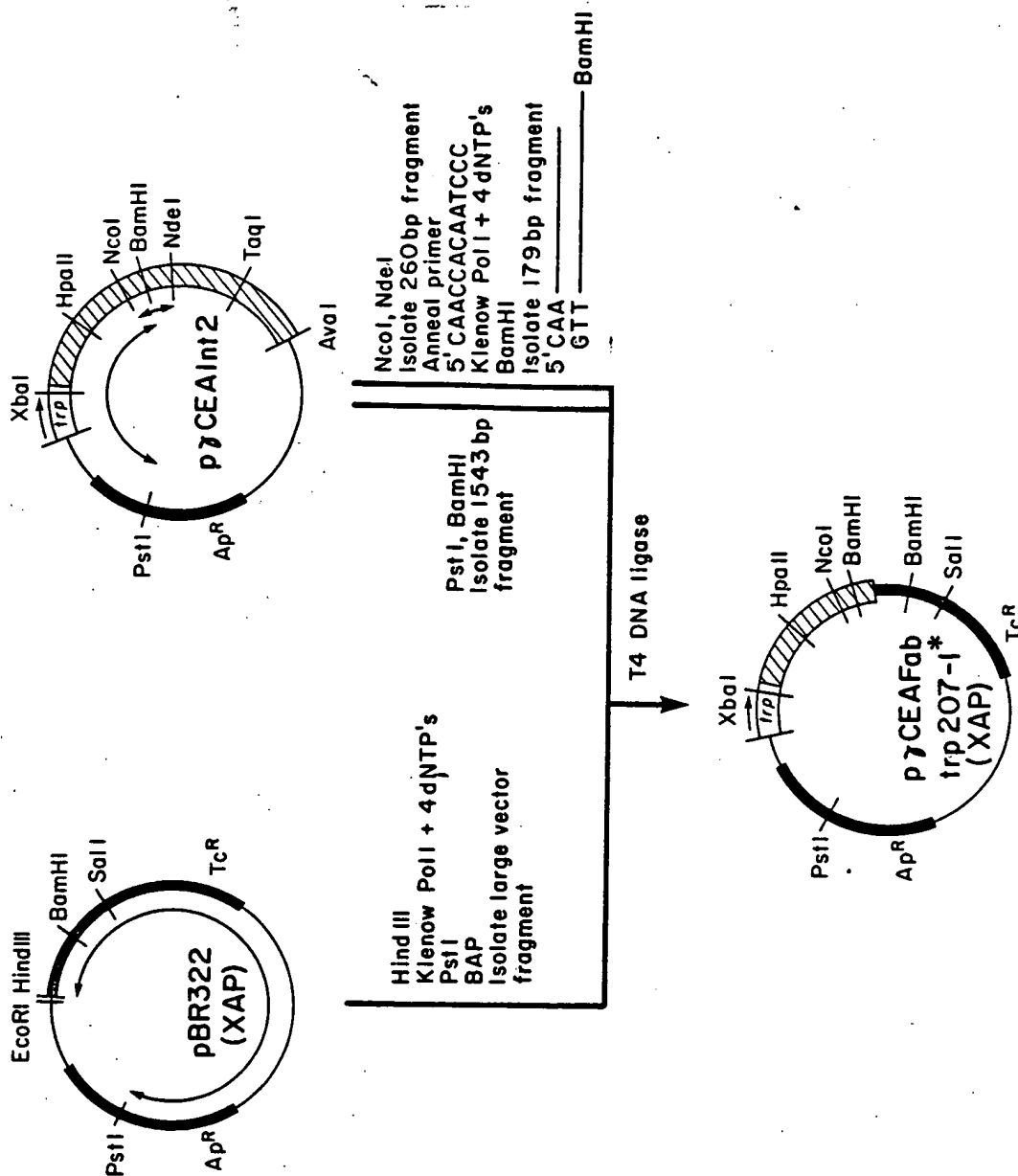


FIGURE 13

Construction of plasmid for direct expression of Fab region from heavy anti CEA chain gene

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